Variation to Cause Host Injury Between Russian Wheat Aphid (Homoptera: Aphididae) Clones Virulent to *Dn4* Wheat

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ABSTRACT Biotypes are infraspecific classifications based on biological rather than morphological characteristics. Cereal aphids are managed primarily by host plant resistance, and they often develop biotypes that injure or kill previously resistant plants. Although molecular genetic variation within aphid biotypes has been well documented, little is known about phenotypic variation, especially virulence or the biotype's ability to cause injury to cultivars with specific resistance genes. Five clones (single maternal lineages) of Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Homoptera: Aphididae), determined to be injurious to wheat, *Triticum aestivum* L., with the *Dn4* gene, were evaluated on resistant and susceptible wheat and barley, *Hordeum vulgare* L., for their ability to cause chlorosis, reduction in plant height, and reduction in shoot dry weight. Variation to cause injury on resistant 'Halt' wheat, susceptible 'Jagger' wheat, and resistant 'STARS-9301B' barley was found among the *Dn4* virulent clones. One clone caused up to 30.0 and 59.5% more reduction in plant height and shoot dry weight, respectively, on resistant Halt than other clones. It also caused up to 29.9 and 55.5% more reduction in plant height and shoot dry weight, respectively, on susceptible Jagger wheat. Although STARS-9301B barley exhibited an equal resistant response to feeding by all five clones based on chlorosis, two clones caused ≈20% more reduction in plant height and shoot dry weight than three other clones. The most injurious clones on wheat were not the most injurious clones on barley. This is the first report of variation to cause varying degrees of plant damage within an aphid biotype virulent to a single host resistance gene. A single aphid clone may not accurately represent the true virulent nature of a biotype population in the field.

KEY WORDS biotype, *Diuraphis noxia*, *Hordeum vulgare*, host plant resistance, *Triticum aestivum*

Insect biotypes are infraspecific classifications based on biological rather than morphological characteristics, and generally morphologically indistinguishable. Many definitions exist, but no single universal definition is applicable to all species and situations. For example, they have been described as an infraspecific category with similar genetic composition for a biological attribute (Saxena and Barrion 1987), or they have been defined by "survival and development on a particular host or by host preference for feeding, oviposition, or both" (Diehl and Bush 1984). Biotypes have been equated with races of pathogenic fungi (Gallun and Khush 1980), and with the aphids, biotypes have even been described as being made up of genetically identical "clones" (Eastop 1973). Many insect species containing biotypes are major pests of grain crops such as wheat, *Triticum aestivum* L.; barley, *Hordeum vulgare* L.; sorghum, *Sorghum bicolor* (L.) Moench; and rice, *Oryza sativa* L., and they are specifically described by their ability to injure crops with host plant resistance genes (Gallun 1977, Saxena and Barrion 1985, Puterka et al. 1992, Porter et al. 1997). Approximately 50% of described biotypes belong to Aphididae (Saxena and Barrion 1987). In cereal aphids and other pests that are largely managed by host resistance, this "virulence" or ability to injure previously resistant plants is the generally accepted criterion for biotype designation.

One aphid pest of wheat and barley that has recently developed biotypes to wheat resistance genes is Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Homoptera: Aphididae). Soon after its introduction into the United States in 1986, an expedited effort to develop Russian wheat aphid resistant wheat and barley cultivars began through the facilitation of the Western Regional Coordinating Committee No. 66 (Souza 1998). The first adapted resistant variety available to U.S. farmers was 'Halt' and it was commercially planted on a limited scale in Colorado as early as 1994, but was not readily available until its official registration in 1996 (Quick et al. 1996). The resistance in Halt was reported to be based on a single dominant gene, *Dn4* (Saidi and Quick 1996). *Dn4* is also the source of

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Table 1. Russian wheat aphid clones established in 2003 from field collections and laboratory cultures

Clone	Location	Host	Date	Collector	*Dn4* Virulent
#1[a]	Stillwater, OK	Schuyler barley	27 May 2003	D.W.M.	−
#2[a]	Fort Collins, CO	Wheat, unknown variety	19 May 2003	Frank Peairs	−
#3	Walsh, CO	Prairie Red wheat	20 May 2003	Cynthia Walker	−
#4	Walsh, CO	Above wheat	20 May 2003	Cynthia Walker	+
#5	Walsh, CO	Above wheat	20 May 2003	Cynthia Walker	+
#6	Walsh, CO	Above wheat	20 May 2003	Cynthia Walker	−
#7	Walsh, CO	Above wheat	20 May 2003	Cynthia Walker	+
#8	Lubbock, TX	Elbon rye	27 May 2003	J. Scott Armstrong	+
#9	Bushland, TX	TAM 105 wheat	23 May 2003	Jerry Michels	−
#10	Scottsbluff, NE	Alliance and Halt wheat	27 May 2003	John Thomas and Gary Hein	−
#11	Akron, CO	Wheat, unknown variety	4 June 2003	D.W.M.	−
#12	Banner County, NE	Halt wheat	4 June 2003	John Thomas and Gary Hein	−
#13	Walsh, CO	Prairie Red wheat	22 May 2003	Cynthia Walker	−
#14[a,b]	Fort Collins, CO	Wheat, unknown variety	21 Aug. 2003	Frank Peairs	+
#15[a,c]	Hays, KS	Wheat, unknown variety	2 Nov. 2003	J. P. Michaud	−

[a] Laboratory colony.
[b] Originally collected from Prairie Red wheat in March 2003 near Walsh, CO, and later described as Biotype 2 (Haley et al. 2004a).
[c] Originally collected from wheat on 22 October 2003 at Hays, KS, by Tom Harvey.

Russian wheat aphid resistance in the varieties 'Prairie Red', 'Yumar', 'Prowers', 'Prowers 99′ (Haley 2000), and 'Ankor' (Haley et al. 2004b). In 2003, it was estimated that 25% of the wheat acreage in Colorado were planted to *Dn4* varieties (Peairs et al. 2004). Unfortunately, a biotype virulent to all *Dn4* wheat varieties was found in southeastern Colorado in March 2003 (Haley et al. 2004a). Although it was initially named biotype B (Colorado State University 2003), the *Dn4* injurious biotype is now referred to as Biotype 2 (Haley et al. 2004a) or RWA Biotype 2 (Porter et al. 2005). Biotype 1 refers to the extant population that is noninjurious to *Dn4* wheat, and is called RWA Biotype 1 (Porter et al. 2005).

The designation of biotype as a taxonomic or evolutionary unit is debated. Biotypes have been proposed to be populations in early stages of speciation (Müller 1985, Saxena and Barrion 1987). Selection of fitter genotypes by host plants and resistant cultivars has been proposed to be a mechanism of biotype formation (Bush 1974, Saxena and Barrion 1987). However, Porter et al. (1997) showed no correlation between biotype occurrence and resistance gene deployment in wheat. The biotype concept has problems, largely because the genetics of biotype groups is poorly understood. One way of better understanding the genetic nature, divergence, and origin of insect biotypes is to compare the amount of diversity within biotypes, relative to the amount of variation between biotypes (Black 1993).

The majority of studies on biotype variation have concentrated on describing variation between biotypes. Much less is known about variation within biotypes. Genetic variation between biotypes has been extensively studied in several aphid species and shown significant genotypic divergence between some biotypes (Black et al. 1992, Puterka et al. 1992, Black 1993, Boulding 1998, Vanlerberghe-Masutti and Chavigny 1998, Shufran et al. 2000). Fewer studies on genetic variation within biotypes have been conducted; however, most have shown that molecular genetic variation within biotypes exists (Anstead et al. 2002, Black et al. 1992, Shufran et al. 1992, Vanlerberghe-Masutti and Chavigny 1998). Although genotypic variation within biotypes has been studied, almost no information on phenotypic variation within biotypes exists. Shufran et al. (1992) reported on variability of life history parameters among biotype E clones of greenbug, *Schizaphis graminum* (Rondani). Bush et al. (1989) demonstrated variability among Russian wheat aphid clones (avirulent to *Dn4*, therefore meeting the criteria of Biotype 1) to cause chlorosis on susceptible varieties of wheat. This was the first demonstration of within-biotype virulence variation, even though the population from Texas did not meet the generally accepted definition of a Russian wheat aphid biotype, which is a population that can injure a cultivar that was previously resistant (Burd et al. 2006).

To better characterize Russian wheat aphid populations, we initiated a study to measure phenotypic variation in virulence among clones virulent to *Dn4* wheat. Recent surveys found that Biotypes 1 and 2 predominate in the United States (Puterka et al. 2007). Biotype 3, also virulent to *Dn4* wheat (Burd et al. 2006), has not been found since its first and only collection in 2002 (Puterka et al. 2007). Besides variation in the ability to damage *Dn4* wheat, we also assessed variation for virulence to 'STARS-9301B', a resistant barley line (Mornhinweg et al. 1995). Even though STARS-9301B barley has not been shown to be susceptible to Biotype 2 or 3 (i.e., virulent to *Dn4* wheat), we chose to include it because it is an important resistance source for that crop. STARS-9301B was the germplasm line used in the development of Russian wheat aphid resistant 'Burton' barley (Bregitzer et al. 2005).

Materials and Methods

Russian wheat aphids were collected from Colorado, Kansas, Nebraska, and Texas during 2003 (Table 1). Fifteen clonal colonies (single maternal lineages)

were established in the laboratory, given numeral designations for easier referral, and maintained on 'Schuyler' (susceptible) barley grown in Fuller's earth (Balcones Minerals Corp., Flatonia, TX) and caged in 3.6-cm-diameter by 21-cm-high poly-cast tubes (Cone-Tainer Co., Canby, OR) under clear 3.5-cm-diameter by 21-cm-high plastic chimneys with mesh covered holes for ventilation. The chimney and poly-cast tube were sealed with Parafilm "M" (American Can Co., Greenwich, CT) to minimize the chances of colony contamination. Environmental conditions were 18°C with a photoperiod of 14:10 (L:D) h.

Each Russian wheat aphid clone was determined to be either Biotype 1 or virulent to *Dn4* wheat. Three seeds each of Halt (*Dn4*) and 'Jagger' (susceptible) wheat were planted 2.5 cm deep in Fuller's earth in a single poly-cast tube and covered with a chimney as described above to prevent accidental aphid infestation. When plants reached Zadoks et al. (1974) growth stage 10, the tubes were thinned to one plant for each variety. Each Halt and Jagger seedling was infested with 10 adult Russian wheat aphids from a single clone by placing them directly on the plants with a fine paint brush. Five replications were conducted per clonal colony. When susceptible Jagger plant reached a chlorosis damage scale of 8 or 9 (Webster et al. 1991), i.e., dead, the corresponding Halt plant was rated for leaf rolling and chlorosis (Webster et al. 1991). Russian wheat aphid clones were determined to be avirulent (Biotype 1) if Halt showed no leaf rolling and minimal chlorosis (rating of 1–3), and virulent if Halt showed leaf rolling and extensive chlorosis (rating of 7–9). Within the five replications of each clone, feeding reactions of Halt were consistent. Ten Russian wheat aphid clones were determined to be avirulent, and five clones were determined to be virulent to *Dn4* (Table 1).

The five *Dn4* virulent clones determined above (Table 1) were used to test for variation in the ability to cause feeding damage to wheat and barley. To increase aphid numbers for infestation of plants in the experiment, each clone was transferred to a 15-cm-diameter by 12-cm-high plastic pot containing 15–20 plants of susceptible 'Otis' barley. Each pot was covered with a 14-cm diameter by 30-cm-high clear plastic chimney with fine mesh cloth covering the top and two 9-cm-diameter holes cut in the sides for ventilation. Only aphids reared solely on Otis barley were used to infest experimental plants.

The experiments were conducted in a greenhouse by using a completely randomized design with 10 replications. Each clone was tested on a resistant and susceptible variety of wheat and barley in two separate experiments. Except for the plant species and varieties, each experiment was identical in materials, methods, and design. In the wheat experiment, the *Dn4* resistant variety was Halt, whereas the susceptible variety was Jagger. In the barley experiment, the resistant germplasm line was STARS-9301B (Mornhinweg et al. 1995) and the susceptible variety was 'Morex'.

Seeds were preimbibed by placing them in petri dishes on top of filter papers dampened with double distilled H_2O and kept in the dark at ambient temperature for 48 h. Seeds were then planted, with radicle down, 2.5 cm deep, in processed Fuller's earth in 15-cm-diameter by 12-cm-high plastic pots. Therefore, each pot contained three susceptible and three resistant plants, for a total of six plants per pot. Each pot was covered with a 14-cm-diameter by 30-cm-high clear plastic chimney with fine mesh cloth covering the top and two 9-cm-diameter holes cut in the sides for ventilation. When plants reached Zadoks et al. (1974) growth stage 10, each pot containing six plants (three resistant and three susceptible) was infested with 60 adult or fourth instar Russian wheat aphids of a single clone. Aphids were evenly divided among the six plants by placing 10 aphids at the base of each plant with a fine paint brush. Ten replications of each clone were used, for a total of 50 pots per experiment. Pots were arranged on a table in five rows and 10 columns (columns oriented north to south) with an additional outside border of uninfested pots to prevent border effects of light and air movement. The position of each pot on the table was chosen using a random number generator. Supplemental light (40-W SunStik, Osram Sylvania Products, Inc., Versailles, KY) with a photoperiod of 14:10 (L:D) h was used. Temperature conditions during the experiment ranged from 18.3 to 27.3°C, and averaged 22.8 ± 0.1°C. Plants were watered as needed with a 4.1 g/liter solution of 20–20–20 water soluble fertilizer (Peters Professional, United Industries Corp., St. Louis, MO).

The aphids were allowed to reproduce and feed undisturbed until the majority of susceptible plants (Jagger or Morex) showed extreme chlorosis or were dead, i.e., had a rating of 8 or 9 by using the chlorosis scale of Webster et al. (1991). At this point, the entire experiment was terminated, and all plants were evaluated for height, shoot dry weight, and chlorosis. The degree of chlorosis was determined using the 1–9 scale according to Webster et al. (1991). Data were subjected to analysis of variance (ANOVA) by using the Kenward–Roger adjustment in PROC MIXED with the LSMEANS option (SAS Institute 1999). Within each experiment, data were sorted and analyzed by plant variety to assess for variation among clones.

Results

Wheat. A significant variety effect was found for chlorosis and shoot dry weight (Table 2). A significant clone effect and clone by variety interaction was found for chlorosis, plant height, and dry weight (Table 2), indicating that some clones were more injurious than others depending on the wheat variety. Mean separations by wheat variety identified which clones were more injurious than others (Table 3).

Variation between *Dn4* virulent clones in the ability to injure Halt was found. Differences between the five clones were found for chlorosis, plant height and shoot dry weight (Table 3). Based on these three parameters, the most damaging was Clone #4 from Walsh,

Table 2. Output from PROC MIXED with Kenward–Roger adjustment (SAS Institute 1999) for plant reactions for two cultivars of wheat (Halt and Jagger) and two cultivars of Barley (STARS-9301B and Morex) after feeding by five *Dn4* virulent Russian wheat aphid clones

Host species	Fixed effect	Num df	Den df	Chlorosis		Plant ht		Shoot dry wt	
				F	*P* > F	*F*	*P* > F	*F*	*P* > *F*
Wheat	Clone	4	44	19.38	<0.0001	15.85	<0.0001	21.07	<0.0001
	Variety	1	44	180.64	<0.0001	0.00	0.9625	19.21	<0.0001
	Clone × variety	4	44	6.85	0.0002	2.55	0.0523	3.48	0.0087
Barley	Clone	4	20	0.61	0.6622	4.70	0.0077	2.34	0.0904
	Variety	1	20	1,446.01	<0.0001	514.20	<0.0001	312.82	<0.0001
	Clone × variety	4	20	0.65	0.6356	4.29	0.0063	3.49	0.0255

CO. On Halt, it induced more chlorosis and a greater reduction in plant height and shoot dry weight than three others collected in Colorado and Clone #8 from Lubbock, TX. Clone #14, used to describe Biotype 2 by Haley et al. (2004a), caused less injury than Clone #4.

Clonal variation for injuriousness was also observed on susceptible Jagger for all plant responses (Table 3). Plant responses of chlorosis, plant height, and shoot dry weight by infestation of Clone #4 were similar on Halt and Jagger (Table 3). Clone #4 was more damaging than some others, especially Clones #5 and #7. On Jagger, Clone #4 was equally as damaging as Clone #14 (Table 3). However, relative ability of *Dn4* virulent clones to cause damage was different on the two wheat varieties. A distinct clone-by-plant variety interaction existed (Tables 2 and 3).

Barley. A significant variety effect was found for chlorosis, plant height, and shoot dry weight (Table 2). A significant clone effect for plant height and a clone by variety interaction for plant height and dry weight was found (Table 2), indicating that some clones were more injurious than others depending on the barley variety. Mean separations by barley variety (or germplasm line) identified which clones were more injurious than others (Table 4).

No variation in the ability to cause injury on resistant STARS-9301B barley was found for chlorosis between the five clones (Table 4). All levels of chlorosis indicated that STARS-9301B was resistant to *Dn4* virulent Russian wheat aphids. However, Clone #7 from Walsh, CO, and Clone #8 from Lubbock, TX, both caused about a 20% reduction in plant height compared with the other three clones (Table 4). A corresponding loss in shoot dry weight by these two clones was also found on STARS-9301B. When Morex barley was fed upon by the same five clones, no variation in any plant responses between clones was found (Table 4). All clones were equally injurious to susceptible Morex barley.

Discussion

All five *Dn4* virulent Russian wheat aphid clones used in this experiment severely damaged Halt wheat, which carries the *Dn4* resistance gene. Chlorosis ratings were 7 or higher for each clone, and all caused leaf rolling. However, among these clones, we detected significant variation in their ability to cause plant injury. On Halt wheat, the most injurious was Clone #4 from Walsh, CO (Table 3). This clone was more damaging than the original Biotype 2 clone (Haley et al. 2004a), which is referred to as Clone #14 in this article. The increased degree of virulence of Clone #4 on Halt for chlorosis, reduction in plant height, and reduction in shoot dry weight was 0.7–13.7, 17.3–30.0, and 42.7–59.5%, respectively. This was also found on susceptible Jagger wheat (Table 3). Clone #4 also caused more injury on Jagger than two other clones (Clones #5 and #7) that were both also collected at the same location. The increased degree of virulence of Clone #4 on Jagger for chlorosis, reduction in plant height, and reduction in shoot dry weight was 2.9–14.6, 12.0–29.9, and 28.1–55.3%, respectively. However, Clones #5 and #7 were less injurious to susceptible Jagger than other *Dn4* virulent clones. A similar

Table 3. Mean ± SE plant responses to feeding by *Dn4* virulent Russian wheat aphids on Halt (*Dn4*) wheat and Jagger (susceptible) wheat

Variety	Clone	Chlorosis[a,b]	Plant ht (cm)[b]	Shoot dry wt (mg)[b]
Halt	#4 Walsh, CO	8.10 ± 0.12a	16.32 ± 0.66c	12.61 ± 1.36c
	#5 Walsh, CO	7.53 ± 0.12b	21.24 ± 0.66ab	22.00 ± 1.36b
	#7 Walsh, CO	6.99 ± 0.13c	23.30 ± 0.69a	31.12 ± 1.43a
	#8 Lubbock, TX	7.43 ± 0.12b	19.73 ± 0.66b	22.20 ± 1.36b
	#14 Fort Collins, CO[c]	7.23 ± 0.12bc	19.95 ± 0.66b	22.49 ± 1.36b
Jagger	#4 Walsh, CO	8.93 ± 0.10a	16.21 ± 0.69c	10.93 ± 1.68c
	#5 Walsh, CO	7.97 ± 0.10c	23.12 ± 0.69a	23.36 ± 1.68a
	#7 Walsh, CO	7.63 ± 0.11c	22.71 ± 0.72ab	24.47 ± 1.76a
	#8 Lubbock, TX	8.50 ± 0.10b	20.52 ± 0.69b	17.39 ± 1.68b
	#14 Fort Collins, CO[c]	8.67 ± 0.10ab	18.15 ± 0.69c	15.21 ± 1.68bc

[a] Rating scale of 1 to 9, where 1 is no damage and 9 is dead plant (Webster et al. 1991).
[b] For each variety, means within columns with the same letter are not significantly different ($P > 0.05$; Tukey–Kramer) (Westfall et al. 1999).
[c] Laboratory colony originally established from aphids collected in Walsh, CO, and later described as Biotype 2 (Haley et al. 2004a).

Table 4. Mean ± SE plant responses to feeding by *Dn4* virulent Russian wheat aphids on STARS-9301B (resistant) and Morex (susceptible) barley

Variety	Clone	Chlorosis[a,b]	Plant ht (cm)[b]	Shoot dry wt (mg)[b]
STARS-9301B	#4 Walsh, CO	2.20 ± 0.28a	34.15 ± 1.35a	50.10 ± 3.56a
	#5 Walsh, CO	2.13 ± 0.28a	33.62 ± 1.35a	47.29 ± 3.56a
	#7 Walsh, CO	2.20 ± 0.28a	26.09 ± 1.35b	32.45 ± 3.56b
	#8 Lubbock, TX	2.20 ± 0.28a	25.67 ± 1.35b	33.18 ± 3.56b
	#14 Fort Collins, CO[c]	2.20 ± 0.28a	31.57 ± 1.35a	43.25 ± 3.56ab
Morex	#4 Walsh, CO	8.20 ± 0.28a	15.59 ± 1.35a	11.56 ± 3.56a
	#5 Walsh, CO	8.47 ± 0.28a	15.14 ± 1.35a	12.78 ± 3.56a
	#7 Walsh, CO	8.53 ± 0.28a	14.60 ± 1.35a	10.33 ± 3.56a
	#8 Lubbock, TX	9.00 ± 0.28a	13.43 ± 1.35a	09.71 ± 3.56a
	#14 Fort Collins, CO[c]	8.80 ± 0.28a	15.65 ± 1.35a	13.15 ± 3.56a

[a] Rating scale of 1 to 9, where 1 is no damage and 9 is dead plant (Webster et al. 1991).
[b] For each variety, means within columns with the same letter are not significantly different ($P > 0.05$; Tukey–Kramer) (Westfall et al. 1999).
[c] Laboratory colony originally established from aphids collected in Walsh, CO, and later described as Biotype 2 (Haley et al. 2004a).

result was observed by Bush et al. (1989) in virulence within Biotype 1 on susceptible wheat.

There was no correspondence between wheat and barley for a clone's ability to cause damage. Clones most injurious to wheat were not necessarily the most injurious to barley. Although Biotype 1 resistant STARS-9301B barley clearly was resistant to all *Dn4* virulent clones, based on chlorosis ratings and no leaf rolling, we detected variation in growth parameters when fed upon by *Dn4* virulent clones (Table 4). Clones #7 and #8 both caused more reduction in plant height and corresponding shoot dry weight (≈20%) than the other clones. Puterka et al. (2006) also found differences in resistant barley plant height among Russian wheat aphid biotypes, even though there were no differences in chlorosis. However, field tests have shown yield reduction in susceptible barley to be the result of leaf rolling and the subsequent trapping of spikes (Mornhinweg et al. 2006). Whether the increased amount of plant mass reduction we observed in the seedling stage would have an impact on subsequent barley grain yield is not known. Currently, reduction in plant height is not a character used in naming Russian wheat aphid biotypes.

To reduce the possible effects of fecundity differences between within and between aphid clones, our experiment allowed free movement of aphids from plant to plant within each pot. This helped assure a uniform distribution of aphid numbers among plants to get an accurate assessment of plant response (Webster et al. 1987). The variation between clones we found can likely be attributed to inherent biological differences among clones in the ability to injure not only *Dn4* wheat, but susceptible wheat as well. Under field production settings, differences between *Dn4* virulent clones to cause injury to wheat may not be economically important, as aphid clonal diversity on single plants has been found to be extensive (Birch et al. 1994, Shufran et al. 1991, Wynne et al. 1994). It is likely that multiple clones and biotypes of Russian wheat aphid infest individual wheat plants in the field. In our biotype determination, we found Biotype 1 (noninjurious to *Dn4* wheat) clones on Prairie Red (*Dn4*) wheat that was showing severe injury symptoms (Table 1). Although five clones caused extreme chlorosis to *Dn4* resistant wheat, the variability to cause more or less injury we found among the clones raises questions about the origin and development of Biotypes 2 and 3, both virulent to *Dn4* wheat (Burd et al. 2006).

It was very interesting to have found a *Dn4* virulent aphid in Lubbock, TX, at the same time Biotype 2 was first discovered in Walsh, CO. No Russian wheat aphid resistant wheat was ever grown near Lubbock, TX, which is ≈480 km from Walsh, CO. These two locations are separated by a large expanse of nonirrigated range land in which no wheat is cultivated, which may act as a barrier to aphid movement. Furthermore, Burd et al. (2006) collected a *Dn4* virulent clone (later named Biotype 3) from this same area in TX during May 2002, a year before the initial discovery of Biotype 2 in Walsh, CO. The observance of *Dn4* virulent aphids outside the area that resistant *Dn4* wheat was grown casts doubt that Biotype 2's occurrence was due to a simple selection by resistant varieties in a way that would be analogous to pesticide resistance.

Molecular genetic studies showed that *S. graminum* biotypes were made up of multiple clones and genotypes (Shufran et al. 1992, Anstead et al. 2002). Variation in aphid life history characters also was found within a single *S. graminum* biotype (Shufran et al. 1992); however, the authors did not investigate variability to cause injury on a single host cultivar. Although our methods could not differentiate between Russian wheat aphid Biotypes 2 and 3, it is likely that most or all *Dn4* virulent clones in this study could be classified as Biotype 2. Surveys since 2003 have only found Russian wheat aphid Biotypes 1 and 2 in the United States (Puterka et al. 2007). The results presented herein are the first to show the ability of different aphid clones to cause varying degrees of plant damage within a biotype virulent to a single plant resistant gene. One aphid clone may not accurately represent the true virulent nature of a biotype population in the field. It is common for plant breeders to use a single laboratory clone when evaluating germplasm for aphid resistance. To reduce the risk of misleading results, within biotype variability should be

taken into consideration by using more than a single insect colony or population in resistant cultivar development.

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References Cited

Anstead, J. A., J. D. Burd, and K. A. Shufran. 2002. Mitochondrial DNA sequence divergence among *Schizaphis graminum* isolates from non-cultivated hosts: haplotypes and host associations. Bull. Entomol. Res. 92: 17–24.

Birch, A.N.E., B. Fenton, G. Malloch, A. T. Jones, M. S. Phillips, B. E. Harrower, J.A.T. Woodford, and M. A. Catley. 1994. Ribosomal spacer length variability in the large raspberry aphid, *Amphorophora idaei* (Aphidinae: Macrosiphini). Insect Mol. Biol. 3: 239–245.

Black, IV, W. C. 1993. Variation in the ribosomal RNA cistron among host-adapted races of an aphid (*Schizaphis graminum*). Insect Mol. Biol. 2: 59–69.

Black, IV, W. C., N. M. DuTeau, G. J. Puterka, J. R. Nechols, and J. M. Pettorini. 1992. Use of the random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) to detect DNA polymorphisms in aphids (Homoptera: Aphididae). Bull. Entomol. Res. 82: 151–159.

Boulding, E. G. 1998. Molecular evidence against phylogenetically distinct host races of the pea aphid (*Acyrthosiphon pisum*). Genome 41: 769–775.

Bregitzer, P., D. W. Mornhinweg, R. Hammon, M. Stack, D. D. Baltensperger, G. L. Hein, M. K. O'Neill, J. C. Whitmore, and D. J. Fiedler. 2005. Registration of 'Burton' barley. Crop Sci. 45: 1166–1167.

Burd, J. D., D. R. Porter, G. J. Puterka, S. D. Haley, and F. B. Peairs. 2006. Biotypic variation among North American Russian wheat aphid (Homoptera: Aphididae) populations. J. Econ. Entomol. 99: 1862–1866.

Bush, G. L. 1974. The mechanisms of sympatric host race formation in the tree fruit flies (Tephritidae), pp. 3–23. *In* M. J. White [ed.], Genetic mechanism of speciation in insects. Australian and New Zealand Book Company, Sydney, Australia.

Bush, L., J. E. Slosser, and W. D. Worrall. 1989. Variations in damage to wheat caused by Russian wheat aphid (Homoptera: Aphididae) in Texas. J. Econ. Entomol. 82: 466–471.

Colorado State University. 2003. New strain of aphid attacks Colorado's wheat. The Fence Post, June 30: 106–107.

Diehl, S. R., and G. L. Bush. 1984. An evolutionary and applied perspective of insect biotypes. Annu. Rev. Entomol. 29: 471–504.

Eastop, V. F. 1973. Biotypes of aphids, pp. 40–51. *In* A. D. Lowe [ed.], Perspectives in applied biology. Bull. Entomol. Soc. NZ. 2.

Gallun, R. L. 1977. The genetic basis of Hessian fly epidemics. Ann. NY Acad. Sci. 287: 223–229.

Gallun, R. L., and G. S. Khush. 1980. Genetic factors affecting expression and stability of resistance, pp. 63–68. *In* F. G. Maxwell and P. R. Jennings [eds.], Breeding plant resistance to insects. Wiley, New York.

Haley, S. D. 2000. Winter wheat breeding update from CSU. Agronomy News 20(5). Cooperative Extension Service, Colorado State University, Fort Collins, CO.

Haley, S. D., F. B. Peairs, C. B. Walker, J. B. Rudolph, and T. L. Randolph. 2004a. Occurrence of a new Russian wheat biotype in Colorado. Crop Sci. 44: 1589–1592.

Haley, S. D., J. S. Quick, J. J. Johnson, F. B. Peairs, J. A. Stromberger, S. R. Clayshulte, B. L. Clifford, J. B. Rudolph, O. K. Chung, and B. W. Seabourn. 2004b. Registration of 'Ankor' wheat. Crop Sci. 44: 1025–1026.

Mornhinweg, D. W., D. R. Porter, and J. A. Webster. 1995. Registration of STARS-9301B barley germplasm resistant to Russian wheat aphid. Crop Sci. 35: 602.

Mornhinweg, D. W., M. J. Brewer, and D. R. Porter. 2006. Effects of Russian wheat aphid on yield and yield components of Field grown susceptible and resistant spring barley. Crop Sci. 46: 36–42.

Müller, F. P. 1985. Biotype formation and sympatric speciation in aphids (Homoptera: Aphididae). Entomol. Gen. 10: 161–181.

Peairs, F., S. Haley, and J. Johnson. 2004. Russian wheat aphid infestations in Prairie Red. (http://triticum.agsci.colostate.edu/newrwa.pdf).

Porter, D. R., J. D. Burd, K. A. Shufran, J. A. Webster, and G. L. Teetes. 1997. Greenbug (Homoptera: Aphididae) biotypes: selected by resistant cultivars or preadapted opportunists? J. Econ. Entomol. 90: 1055–1065.

Porter, D. R., C. A. Baker, and M. El-Bouhssini. 2005. Resistance in wheat to a new North American-Russian wheat aphid biotype. Plant Breed. 124: 603–604.

Puterka, G. J., J. D. Burd, and R. L. Burton. 1992. Biotypic variation in a worldwide collection of Russian wheat aphid (Homoptera: Aphididae). J. Econ. Entomol. 85: 1497–1506.

Puterka, G. J., J. D. Burd, D. W. Mornhinweg, S. D. Haley, and F. B. Peairs. 2006. Response of resistant and susceptible barley to infestations of five *Diuraphis noxia* (Kurdjumov), (Homoptera: Aphididae) biotypes. J. Econ. Entomol. 99: 2151–2163.

Puterka, G. J., J. D. Burd, D. Porter, K. Shufran, C. Baker, B. Bowling, and C. Patrick. 2007. Distribution and diversity of Russian wheat aphid (Homoptera: Aphididae) biotypes in North America. J. Econ. Entomol. 100: 1679–1684.

Quick, J. S., G. E. Ellis, R. M. Mormann, J. A. Stromberger, J. F. Shanahan, F .B. Peairs, J. B. Rudolph, and K. Lorenz. 1996. Registration of 'Halt' wheat. Crop Sci. 36: 210.

Saidi, A., and J. S. Quick. 1996. Inheritance and allelic relationships among Russian wheat aphid resistance genes in winter wheat. Crop Sci. 36: 256–258.

SAS Institute. 1999. SAS/STAT user's guide, version 8. SAS Institute, Cary, NC.

Saxena, R. C., and A. A. Barrion. 1985. Biotypes of the brown planthopper, *Nilaparvata lugens* (Stål) and strategies in deployment of host plant resistance. Insect Sci. Appl. 6: 271–289.

Saxena, R. C., and A. A. Barrion. 1987. Biotypes of insect pests of agricultural crops. Insect Sci. Appl. 8: 453–458.

Shufran, K. A., W. C. Black, I. V., and D. C. Margolies. 1991. DNA fingerprinting to study spatial and temporal distributions of an aphid, *Schizaphis graminum* (Homoptera: Aphididae). Bull. Entomol. Res. 81: 303–313.

Shufran, K. A., J. D. Burd, J. A. Anstead, and G. Lushai. 2000. Mitochondrial DNA sequence divergence among greenbug (Homoptera: Aphididae) biotypes: evidence for host adapted races. Insect Mol. Biol. 9: 179–184.

Shufran, K. A., D. C. Margolies, and W. C. Black, IV. 1992. Variation between biotype E clones of *Schizaphis graminum* (Homoptera: Aphididae). Bull. Entomol. Res. 82: 407–416.

Souza, E. J. 1998. Host plant resistance to the Russian wheat aphid (Homoptera: Aphididae) in wheat and barley, pp. 122–147. *In* S. S. Quisenberry and F. B. Peairs [eds.], Response model for an introduced pest-the Russian wheat aphid. Proceedings. Thomas Say Publications in Entomology. Entomological Society of America. Lanham, MD.

Vanlerberghe-Masutti, F., and P. Chavigny. 1998. Host-based genetic differentiation in the aphid *Aphis gossypii* Glover, evidenced from RAPD fingerprints. Mol. Ecol. 7: 905–914.

Webster, J. A., C. A. Baker, and D. R. Porter. 1991. Detection and mechanisms of Russian wheat aphid (Homoptera: Aphididae) resistance in barley. J. Econ. Entomol. 84: 669–673.

Webster, J. A., K. J. Starks, and R. L. Burton. 1987. Plant resistance studies with *Diuraphis noxia* (Homoptera: Aphididae), a new United States wheat pest. J. Econ. Entomol. 80: 944–949.

Westfall, R. H., R. D. Tobias, D. Rom. R. D. Wolfinger, and Y. Hockberg. 1999. Multiple comparisons and multiples tests using the SAS system. SAS Institute, Cary, NC.

Wynne, I. R., J. J. Howard, H. D. Loxdale, and C. P. Brooks. 1994. Population genetic structure during aestivation in the sycamore aphid *Drepanosiphum plantanoidis* (Hemiptera: Drepanosiphidae). Eur. J. Entomol. 91: 375–383.

Zadoks, J. C., T. T. Chang, and C. F. Konzak. 1974. A decimal code for the growth stages of cereals. Weed Res. 14: 415–421.

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